CALTE.004CP1 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Dong et al.
Appl. No.	:	09/849,869
Filed	:	November 3, 2000
For	:	PAIN SIGNALING MOLECULES
Examiner	:	John Ulm
Group Art Unit	:	1646

CERTIFICATE OF MAILING

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on

July 12, 2004
(Date)

Michael L. Fuller, Reg. No. 36,516

DECLARATION UNDER 37 C.F.R. §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I David J. Anderson, do hereby declare and say as follows:

1. I am an inventor on the above-referenced application and a professor in the Department of Biology at the California Institute of Technology, Pasadena, California 91125. I directly supervised all of the work described in paragraphs 2-8 below.

2. A subtractive hybridization screen using RNA from wild type mice and mice that were lacking the neurogenin 1 (Ngn1) transcription factor was carried out in my laboratory. The aim of the screen was to identify genes that are specifically expressed in $trkA^+$ nociceptive neurons. The screen was based on the finding that the lack of Ngn1 eliminates the nociceptive subset of neurons in the dorsal root ganglia.

3. Consistent with its design, the screen identified a number of signaling molecules known to be involved in nociceptor function, including VR1, CGRP and SNS-TTXi.

4. The screen also identified several novel genes, one of which we predicted to encode a novel G protein-couple receptor based on sequence analysis. We called this gene *Mas related gene 3* (Mrg3; later called MrgA1) in view of its limited homology with MAS1.

5. We used the Mrg3 gene to identify a number of additional Mrg family members with at least about 50% identity. Seven additional novel murine genes (Mrg4-5 and Mrg7-12; later called MrgA2-8) were identified, as well as two novel human genes (MrgX1 and MrgX2). The amino acid sequence of the MrgX1 receptor is provided in SEQ ID NO:16 of the above-captioned application.

6. Using *in situ* hybridization, expression of all eight murine receptors (Mrg3-5 and Mrg8-12) was examined in mouse dorsal root ganglia (DRG). All eight were found to be expressed in wild type DRG but not in DRG from mice lacking the Ngn1 transcription factor. This result was consistent with the design of the screen and indicated that the Mrg expressing neurons were likely to be nociceptive.

7. *In situ* hybridization was performed on neonatal DRG using probes for Mrg3 and Mrg5 in conjunction with immunolabeling using anti-trkA antibodies. These experiments confirmed that Mrgs were expressed in $trkA^+$ neurons.

8. To determine if Mrgs were expressed in a more restricted subset of nociceptive neurons, *in situ* hybridization was carried out in combination with fluorescent labeling for isolectin B4 (IB4), a well-known marker for a particular subset of nociceptive neurons. The results indicated that Mrgs were expressed in IB4 positive nociceptive neurons.

9. Based solely on the results presented in paragraphs 2-8, it is my considered scientific opinion that MrgX1 is a G protein-coupled receptor that is involved in pain signaling and could be used to identify compounds that modulate pain sensation.

10. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. I declare that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 or Title 18 of the United States

Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 7-12-04 By: _____
David J. Anderson

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